

Pulmonary function changes and immunomodulation of cytokine expression by zafirlukast after sensitization and allergen challenge in brown Norway rats

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Summary

Background The cysteinyl leukotrienes are known important mediators in bronchial asthma.

Objective Our purpose was to evaluate the effect of zafirlukast on the late-phase reaction, bronchial hyper-responsiveness (BHR) and T cell-related cytokine mRNA expression in ovalbumin (OA)-sensitized brown Norway rats (BNRs).

Methods Thirty BNRs were equally divided into three groups. Group I and II animals were sensitized and then provoked with OA. Zafirlukast was given intraperitoneally (i.p.) to group I animals prior to provocation. Group II animals received i.p. normal saline. Group III animals (controls) were not sensitized and breathed aerosolized saline. After OA provocation, the animals were anaesthetized. Pulmonary function tests (PFT) were performed at baseline and after varying doses of acetylcholine. Thereafter, bronchoalveolar lavage (BAL) was performed and the lungs were examined histologically. Total RNA was extracted from lung tissue and reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using primers for IL-2, IL-4, IL-5, IL-6, IL-10, TNF- α , IFN- γ , iNOS and β -actin.

Results Group II OA-treated BNRs had worse PFT results, more severe bronchoconstriction in response to acetylcholine, and more severe inflammation in lung tissue than the other two groups. Group II had higher IL-2, IL-4, IL-10 and IFN- γ cytokine levels in BAL fluid and higher IL-2, IL-4, IL-5, IL-6, IL-10, IFN- γ , TNF- α and iNOS mRNA levels when compared with group I.

Conclusion Zafirlukast is effective in preventing late-phase bronchoconstriction and BHR, reducing inflammatory response, and decreasing IL-2, IL-4, IL-5, IL-6, IL-10 and IFN- γ and iNOS mRNA expression.

Keywords bronchial asthma, bronchial hyper-reactivity, brown Norway rats, maximal forced expiratory manoeuvre, ovalbumin, zafirlukast

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Introduction

Bronchial asthma is a common disease with an increasing prevalence and mortality [1–4]. Experimental models are needed to help investigate this disease. Asthma in brown Norway rats (BNRs) has many similarities to human allergic asthma, as characterized by early- and late-phase reactions, bronchial hyper-responsiveness (BHR) and accumulation of eosinophils and activated T lymphocytes in the airways after allergen challenge. The BNR has thus been widely used as an animal model for asthma [5–7].

Inflammation is related to bronchial asthma, its severity and BHR [8–12]. Th2 but not Th1 lymphocytes have been reported to have proinflammatory roles by expressing and generating cytokines that activate and attract effector cells and release other cytokines and mediators. There is also evidence demon-

strating that Th2 cytokines are increased but Th1 cytokines decreased in the lung tissue of ovalbumin (OA)-sensitized and exposed BNR [8].

Allergen exposure causes bronchoconstriction in patients with asthma and leads to leukotriene production and increased BHR. The cysteinyl leukotrienes (LT), LTC₄, LTD₄ and LTE₄, are known mediators of bronchoconstriction, airway wall oedema and mucous hypersecretion in patients with asthma [13]. Other actions of leukotrienes in asthma include enhancing BHR, histamine release, cytokine release (e.g. IL-5), recruiting inflammatory cells to the airways, and stimulating proliferation of smooth muscle cells and fibroblasts. Bronchoalveolar lavage (BAL) fluid in patients with an asthma attack contains increased numbers of eosinophils, macrophages, and lymphocytes, and increased concentrations of albumin, LTC₄, and other inflammatory mediators [14, 15]. The inflammatory cells are functionally activated [16].

The purpose of this study was to evaluate the effect of zafirlukast on the late-phase reaction and BHR in BNRs following OA sensitization. We also examined its effect on cytokine mRNA expression patterns in BNR lung tissue.

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Materials and methods

Thirty BNRs (weight ranging from 250 to 350 g) were divided into three groups. Each group consisted of 10 weight-matched male animals. The mean weight of group I was 290 ± 26 g, of group II 297 ± 24 g and of group III 294 ± 21 g. Aerosol sensitization and challenge with OA were performed on group I and II animals, while group III animals served as normal controls.

Each group I and II BNR was put into a closed chamber ($30 \times 30 \times 16$ cm plastic box) with two small holes. One served as a gas inlet and the other as a gas outlet. Two mL of 1% ovalbumin (OA) were aerosolized by nebulizer and delivered continuously into the closed chamber with a gas delivery flow of 8 L/min. The animal was exposed to the OA in the chamber for 10 min.

A second sensitization was performed 7 days later using the same procedure. Another 7 days later, a provocation test was performed. Prior to provocation, zafirlukast 1 mg/kg was given intraperitoneally to group I animals and normal saline to group II animals. Thirty minutes later, 3 mL of 4% aerosolized OA were delivered by nebulizer for 10 min into the closed chamber.

Control animals in group III breathed aerosolized saline rather than OA under the same conditions as the other animals.

Study protocol, procedure and pulmonary function tests

Pulmonary function and acetylcholine provocation tests were carried out under general anaesthesia (sodium pentobarbitone, 50 mg/kg via intraperitoneal injection), using whole-body plethysmographic methods as previously described in detail [17, 18]. For groups I and II, this was done 24 h after the OA provocation test, and for group III, 24 h after the final saline aerosolization. Animals were then paralysed with an intravenous injection of gallamine triethiodide (4 mg/kg) and artificially ventilated with a small animal ventilator at a tidal volume of 6 mL/kg and a frequency of 60 breaths/min. All animals were stable without spontaneous breathing 5 min after gallamine was given. Pulmonary function tests (PFT) including airway opening pressure (PaO) during ventilator breathing, total respiratory system compliance (Crs), total respiratory system resistance (Rrs) and a maximal forced expiratory maneuver (MFEM) were measured at baseline. Thereafter, 25, 50, 75 and 100 μ g/kg of acetylcholine (Ach) were given intravenously at 30-min intervals. PFTs were done 5 s after each dose of acetylcholine. Before each successive dose of Ach, the flow volume loop returned to baseline.

For the MFEM, the lungs were inflated to total lung capacity (TLC, lung volume at PaO = 30 cm H₂O) three times; a solenoid valve regulated the inflation. At peak volume during the third inflation, the inflation valve was shut off and another solenoid valve was immediately opened for deflation. The deflation valve was connected to a 20-L container with a pressure of -30 cm H₂O (subatmospheric). The negative pressure of 30 cm H₂O produced maximal expiratory flow ($V_{\max}$). The parameters of the flow volume loop (FV loop), including peak flow (the maximal flow rate of the FV loop), MFEF 75% (the flow rate at 75% TLC), MFEF 50% (the flow rate at 50% TLC) and MFEF 25% (the flow rate at 25% TLC) were recorded [17, 18].

PD₂₀MFEF 50%Ach was the dose of acetylcholine required to produce a decrease in MFEF 50% of 20% from baseline, and

PD₂₀MFEF 25%Ach the dose required to produce a decrease in MFEF 25% of 20%.

Bronchoalveolar lavage (BAL)

After completion of the pulmonary function tests, BAL was performed twice using 10 mL normal saline (total 20 mL). The BAL fluid was collected into plastic flasks and then strained through one layer of surgical gauze and centrifuged at 500 g for 5 min. The cell pellet was washed three times with sterile saline solution and re-suspended in RPMI-1640. A small portion was taken for evaluation of cell number and viability, as assessed by trypan blue exclusion.

Cytokine levels in BAL fluid

The concentrations of IL-2, IL-4, IL-10 and IFN- γ in the BAL fluid were measured in duplicate, in a blinded fashion, using a commercially available rat-specific IL-2, IL-4, IL-10 and IFN- γ ELISA kit (R & D, Minneapolis, MN, USA) with a detection limit of < 10 pg/mL for IL-2, < 5 pg/mL for IL-4, < 10 pg/mL for IL-10 and < 10 pg/mL for IFN- γ . A microplate reader (Spectra Reader, SLT Lab Instruments, Grödig, Austria) was used to measure absorbance at 450 nm. Background absorbance of blank wells was subtracted from the standards and unknowns before determination of the sample concentrations. Total protein was measured using a commercial reagent (Bio-rad, Hercules, CA, USA) and concentration was calculated using a standard curve constructed with bovine serum albumin (Bio-rad).

Histological examination

After BAL, each animal's chest was opened and the lungs were removed. Histological specimens (six specimens per animal consisting of trachea and five lobes of the lung) were prepared. Specimens were fixed in 10% formaldehyde, dehydrated in different concentrations of alcohol, embedded in paraffin, cut into 4- μ m thickness, stained with haematoxylin and eosin, and examined by light microscope for evaluation of the severity of inflammation. Each specimen was blindly assigned an inflammation score by two pathologists as follows: 1 = rare or occasional inflammation cells scattered through the lung or trachea; 3 = abundant inflammatory cells scattered through the lung or trachea; 2 = inflammatory cells between the levels of 1 and 3. The total inflammation score for each animal was calculated as the mean of the scores for the five lung sections and trachea.

Cytokine mRNA analysis

Total RNA was extracted from lung tissue samples by a method described previously [19] and RT-PCR was performed using an RT for PCR kit (Clontech, Palo Alto, CA, USA). The following T cell-related cytokines were evaluated by measuring their mRNA expression: IL-2, IFN- γ (Th1-related) and IL-4, IL-5 and IL-10 (all Th2-related) and iNOS with a PCR kit (Kevin Science Technology, Brea, CA, USA).

Preparation of cDNA and PCR analysis

Briefly, two micrograms of RNA in a 25- μ L volume were first primed with 2 μ L oligo-(dT)₁₈ primer, 20 μ M, at 70 °C, for 2 min and then kept on ice for 5 min. After the reaction, a mixture containing 8 μ L of 5X reaction buffer, 2 μ L dNTP mix 10 μ M (each), 1 μ L recombinant RNase inhibitor, 40 units/ μ L, and 2 μ L MMLV reverse transcriptase, 200 units/ μ L, was added to

synthesize cDNA. cDNA was amplified by 40 PCR cycles in a thermal cycler (Hybaid Omnigene, Ashford, Middlesex, UK). The first 20 cycles of PCR were under a touch down method consisting of a denaturation step (94 °C for 1 min), an annealing step (65 to 55 °C for 1 min, 0.5 °C/cycle touch down, 55 °C for 20 cycles), and an extension step (72 °C for 1 min). The following 20 cycles of PCR were then amplified at a fixed annealing temperature 55 °C. During the last cycle the 72 °C step was extended to 5 min. The PCR product was analysed with 2% agarose gel electrophoresis. The identity of each cytokine cDNA was determined by the PCR product obtained by electrophoresis, and confirmed by DNA sequencing of the PCR products. The quantities of target messages were determined where the ratio of target band intensities were analysed by equations of the β -actin line [19].

Data analysis

Student's *t*-test and ANOVA test were used for statistical analysis where appropriate. If ANOVA test showed statistical significance, the Scheffe test was also done. All values were expressed as the mean $\pm$ standard deviation, with significance accepted when $P < 0.05$.

Results

Pulmonary function test data

The mean data at baseline of peak flow, MFEF 75%, MFEF 50% and MFEF 25% of the flow volume loop for all three groups are shown in Table 1. There was no difference in peak flow, MFEF 75%, PaO and TLC among the three groups, but the MFEF 50% and MFEF 25% were lower in group II than in both group I and group III ($P < 0.05$). There was no difference between group I and group III (Table 1).

The percentages of change of MFEF 50%TLC and MFEF 25%TLC in group II (Figs 1a and b) were higher than in group I and group III at doses of acetylcholine higher than 25 μ g/kg. There was no difference between the percentage change of MFEF 50%TLC and MFEF 25%TLC between group I and group III at each doses of acetylcholine. The PD₂₀ Ach MFEF 50% and MFEF 25% were significantly ($P < 0.001$) higher in group I than in group II but lower than in group III

Table 1. Comparison of mean peak flow, MFEF 75%, MFEF 50%, MFEF 25%, total respiratory system compliance (Cr_s), airway opening pressure (PaO) at tidal breathing and total lung capacity (TLC) among all groups at baseline

	Group I	Group II	Group III
Peak flow (mL/s)	104.2 $\pm$ 4.4	103.7 $\pm$ 5.1	102.5 $\pm$ 4.9
MFEF 75% (mL/s)	102.4 $\pm$ 4.5	101.1 $\pm$ 4.3	100.4 $\pm$ 5.1
MFEF 50% (mL/s)	84.8 $\pm$ 4.1	72.4 $\pm$ 4.8*	85.6 $\pm$ 4.7
MFEF 25% (mL/s)	44.7 $\pm$ 4.6	32.1 $\pm$ 4.5*	47.8 $\pm$ 4.7
Cr _s (mL/cmH ₂ O)	0.46 $\pm$ 0.05	0.43 $\pm$ 0.05	0.47 $\pm$ 0.06
PaO (cmH ₂ O)	3.5 $\pm$ 0.5	3.8 $\pm$ 0.5	3.4 $\pm$ 0.5
TLC (mL)	14.3 $\pm$ 0.5	14.1 $\pm$ 0.5	14.6 $\pm$ 0.5

Data are presented as mean $\pm$ SD. Scheffe test was used. *The comparison of MFEF 25% and MFEF 50% between group I and II, and group II and III, showed significant difference ($P < 0.05$).

for each of the following parameters: MFEF 50% (118 μ g, 26 μ g, > 500 μ g) and MFEF 25% (77 μ g, 12 μ g, > 100 μ g) ($P < 0.05$) (Figs 1a and b).

Bronchoalveolar lavage

There was no difference in the recovery rate of BAL fluid among the three groups (84.7 $\pm$ 2.5% vs. 86.2 $\pm$ 2.8% vs. 85.4 $\pm$ 3.1%). Group II had higher total cell counts and higher absolute counts of each cell type than the other two groups. The percentage of eosinophils and lymphocytes in group II was higher than that of the other two groups, while the percentage of macrophages was lower in group II (Table 2). Group I had higher total cell counts and higher absolute counts of each cell type than group III. The percentage of eosinophils and lymphocytes in group I was higher than that of group III, while the percentage of macrophages was lower in group I than in group III (Table 2). Group II had higher IL-2, IL-4, IL-10 and IFN- γ cytokine and total protein levels in BAL fluid when compared with the group I zafirlukast pre-treated BNR (Table 3). Groups I and II had higher IL-4 and IL-10 cytokine and total protein levels but lower IL-2 and IFN- γ cytokine levels in BAL fluid when compared with group III normal controls (Table 3).

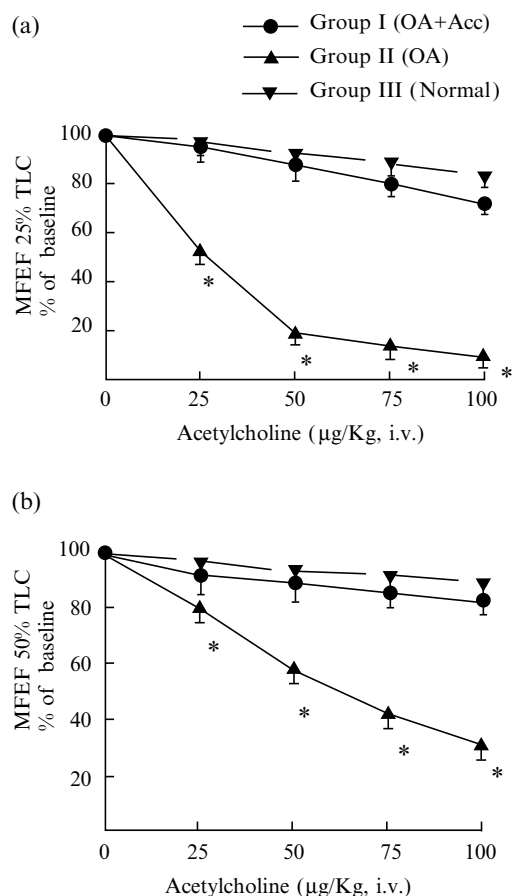


Fig. 1. Dose response curves of maximal forced expiratory flow in groups I, II and III. (a) The percentage change of MFEF 25% from baseline in group II is higher than in groups I and III when acetylcholine doses higher than 25 μ g/kg were given. (b) The percentage of change of MFEF 50% from baseline in group II is higher than in groups I and III when acetylcholine doses higher than 25 μ g/kg were given.

Table 2. Total differential cell counts in BAL fluid

	Group I	Group II	Group III
Total cell count (To10 ⁴ /mL)	4.2 ± 0.5*	9.3 ± 1.2†	0.8 ± 0.2‡
Macrophages			
Cell count	3.4 ± 0.5*	5.8 ± 0.6†	0.72 ± 0.03‡
Percentage of total cell count (%)	81.1 ± 4.6*	62.7 ± 4.9†	89.5 ± 2.8‡
Lymphocytes			
Cell count	0.34 ± 0.1*	1.46 ± 0.3†	0.04 ± 0.01‡
Percentage of total cell count (%)	8.2 ± 2.3*	15.7 ± 3.4†	4.6 ± 1.7‡
Neutrophils			
Cell count	0.0 ± 0.02*	0.13 ± 0.04†	0.01 ± 0.003‡
Percentage of total cell count (%)	1.5 ± 0.4	1.4 ± 0.4	1.6 ± 0.5
Eosinophils			
Cell count	0.39 ± 0.13*	1.88 ± 0.43†	0.03 ± 0.01‡
Percentage of total cell count (%)	9.2 ± 3.8*	20.2 ± 3.6†	4.3 ± 2.1‡

BAL = bronchoalveolar lavage; data are presented as mean ± SD. Scheffe test was used. *Differences between groups I and III were significant for all parameters except percentage of neutrophils ($P < 0.05$). †Differences between groups I and II were significant for all parameters except percentage of neutrophils ($P < 0.05$). ‡Differences between groups II and III were significant for all parameters except percentage of neutrophils ($P < 0.05$).

Table 3. Cytokine levels in BAL fluid

	Group I	Group II	Group III
IL-2 (pg/mL)	16.1 ± 3.1*	23.2 ± 3.6†	31.2 ± 4.1‡
IL-4 (pg/mL)	14.3 ± 2.8*	21.2 ± 3.4†	8.6 ± 2.6‡
IL-10 (pg/mL)	20.1 ± 3.3*	34.2 ± 4.1†	13.4 ± 3.1‡
IFN-IF (pg/mL)	8.1 ± 2.6*	15.4 ± 4.1†	32.4 ± 5.8‡
Total protein (pg/mL)	12.4 ± 4.5*	27.1 ± 5.7†	5.6 ± 2.9‡

BAL = bronchoalveolar lavage; data are presented as mean ± SD. Scheffe test were used. *Differences between groups I and III were significant for all parameters ($P < 0.05$). †Differences between groups I and II were significant for all parameters ($P < 0.05$). ‡Differences between groups II and III were significant for all parameters ($P < 0.05$).

Histology of lung tissue

There was only mild hyperaemia, interstitial oedema and inflammatory cell infiltration in the lung tissue and bronchi of group I (Fig. 2a). In contrast, the lung tissue and bronchi of group II OA-sensitized BNRs demonstrated severe inflammation, characterized by hyperaemia, interstitial oedema and inflammatory cell (especially lymphocyte and eosinophil) infiltration (Fig. 2b). There was absence of hyperaemia, interstitial oedema and inflammatory cell infiltration in the lung tissue and bronchi of group III (Fig. 2c). The mean inflammatory scores in group II were higher than in the other two groups (1.3 ± 0.4 vs. 2.5 ± 0.03 vs. 0.3 ± 0.1) ($P < 0.05$).

Cytokine mRNA profile using RT-PCR

The mean ratios of IL-4, IL-5, IL-6, IL-10, TNF- α and iNOS mRNA levels relative to β -actin in group II were higher than in either group I or III (Figs 3 and 4). The mean ratios of IFN- γ and IL-2 were significantly lower in group I than in group II but higher in group III than in group II (Fig. 4). The mean ratios of

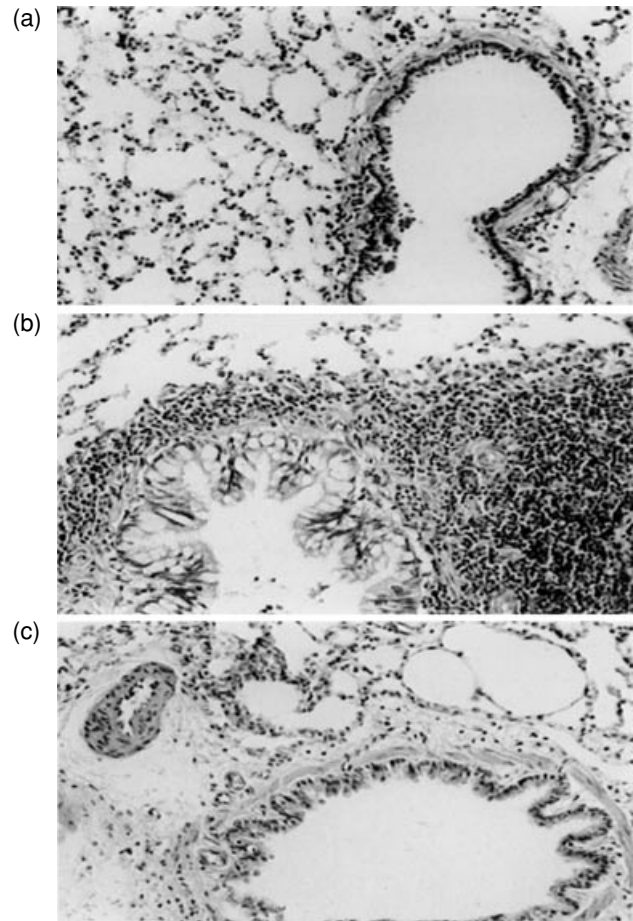


Fig. 2. Histology of lung tissue and bronchi (HE stain 200 $\times$). (a) From a group I animal, demonstrating mild inflammation characterized by mild hyperaemia, interstitial oedema and inflammatory cell infiltration. (b) From a group II animal, demonstrating severe inflammation, characterized by hyperaemia, interstitial oedema and inflammatory cell infiltration. There is also evidence of bronchial epithelial cell desquamation. (c) From a group III animal, demonstrating absence of hyperaemia, interstitial oedema and inflammatory cell infiltration.

IL-4, IL-5, IL-6, IL-10, TNF- α and iNOS mRNA levels relative to β -actin in group I were higher than those in group III. The mean ratios of IFN- γ and IL-2 were significantly higher in group III than in group I (Figs 3 and 4). Figure 5 shows an example of these results from a group I, group II and group III BNR. The group II animal had higher IL-4, IL-5, IL-6, IL-10, iNOS, IFN- γ and TNF- α mRNA expression compared with the group I zafirlukast-pretreated BNR. The group I OA treated BNR had higher IL-2, IL-4, IL-5, IL-6, IL-10, iNOS and TNF- α mRNA expression but lower IL-2 and IFN- γ mRNA expression compared with the group III normal control.

Discussion

Lipoproteins, mucoproteins and other materials can interfere with the measurement of cytokine levels in BAL fluid. In this study, total protein was measured using a commercial reagent (Bio-rad) and concentrations were calculated using a standard curve constructed with bovine serum albumin (Bio-rad).

In this study, we demonstrated that zafirlukast pre-treatment resulted in decreased BHR and a much less severe inflammatory

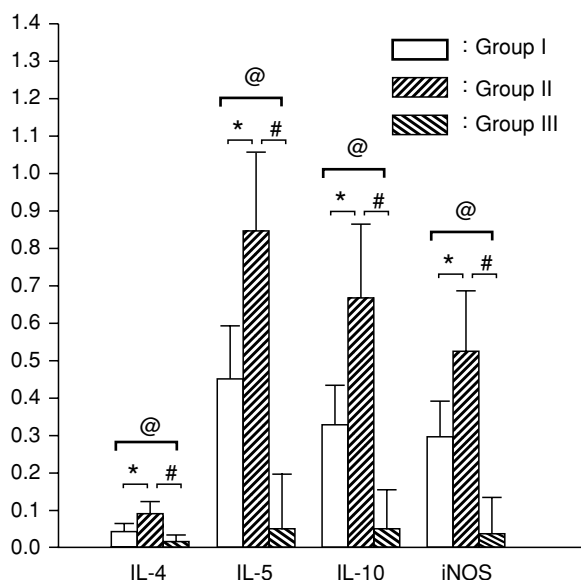


Fig. 3. Mean ratios of IL-4, IL-5, IL-10 and iNOS mRNA levels to β -actin in groups I and III were lower than in group II, mean ratios of IL-4, IL-5, IL-10 and iNOS mRNA levels to β -actin in groups I were higher than in group III as measured by densitometry. * $P < 0.05$ comparison between groups I and II. # $P < 0.05$ comparison between groups II and III. @ $P < 0.05$ comparison between groups I and III. Scheffe test.

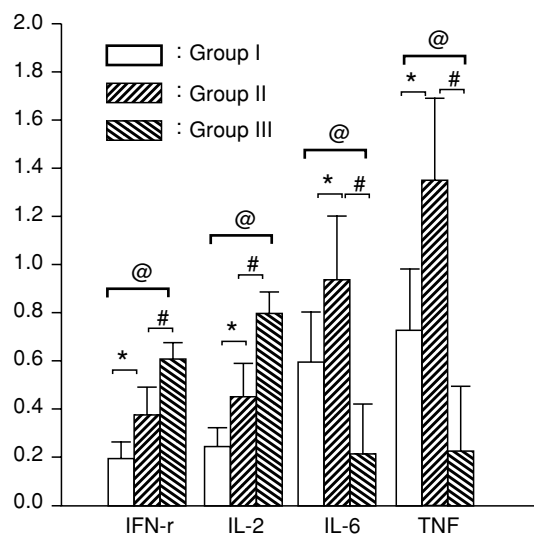


Fig. 4. Expression of IFN- γ , IL-2, IL-6 and TNF was significantly reduced in group I compared with group II (* $P < 0.05$). Expression of IFN- γ and IL-2 was significantly reduced in group I compared with group III. Expression of IL-6 and TNF was significantly increased in group I compared with group III. (@ $P < 0.05$). Expression of IFN- γ and IL-2 was significantly reduced in group II compared with group III. Expression of IL-6 and TNF was significantly increased in group II compared with group III (# $P < 0.05$). Scheffe test.

response after OA challenge than that which occurred in untreated sensitized rats. The group I zafirlukast-pre-treated BNRs had decreased BAL fluid IFN-cytokine levels when compared with the group II OA-treated BNRs. The group II OA-treated BNRs had lower IFN- γ cytokine levels in BAL fluid

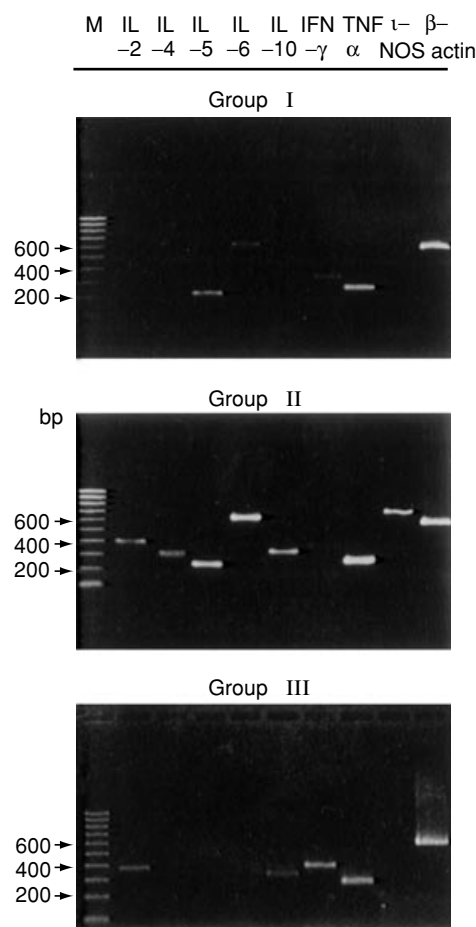


Fig. 5. (a) A group II OA-treated BNR had increased IL-2, IL-4, IL-5, IL-6, IL-10, iNOS, IFN- γ and TNF- α mRNA expression compared with a group I zafirlukast-pretreated BNR. (b) A group II OA-treated BNR had increased IL-4, IL-5, IL-6, IL-10, iNOS and TNF- α m-RNA expression but decreased IL-2 and IFN- γ mRNA expression compared with a group III normal control.

when compared with group III normal controls. The mean ratios of IL-6 and TNF- α , IFN- γ , and the iNOS mRNA levels relative to β -actin in group I was lower than those in group II. It is possible that zafirlukast affects expression of IL-6, TNF- α , IFN- γ or iNOS, which modulate the inflammatory reaction.

IL-4, IL-5 and IL-10 are Th2 related cytokines. Other cellular sources of IL-4 and IL-5 are mast cells, and IL-10 are CD8T cells, B lymphocytes and macrophages. IL-4 has been related to increases in serum IgE. IL-5 is thought to be a potent eosinophil chemotactic factor. Many studies have demonstrated a potential link between cysteinyl leukotrienes and IL-5. Recent work has also demonstrated that LTD4-induced airway eosinophilia in guinea-pigs can be blocked by rat anti-mouse IL-5 monoclonal antibody. This suggests an indirect mechanism of LTD4 on eosinophil recruitment through the action of IL-5. IL-10 is a regulatory cytokine that has been suggested for treatment of asthma because of its immunosuppressive and anti-inflammatory properties. In contrast to these suggestions, IL-10 concentrations in BAL fluid and IL-10 mRNA in lung tissue were significantly higher in allergic subjects with asthma than in control subjects and non-allergic subjects with asthma [20]. Makela et al. also demonstrate in a model of allergic

sensitization that mice deficient in IL-10 (IL-10^{-/-}) develop a pulmonary inflammatory response but fail to exhibit airway hyper-responsiveness in both *in vitro* and *in vivo* assessments of lung function. Reconstitution of these deficient mice with the IL-10 gene fully restores development of airway hyper-responsiveness comparable with control mice. Their results indicate an important role of IL-10 in regulating the tone of the airways after allergic sensitization and challenge [21].

There is now evidence that the interaction between RSV and the monocytic antigen-presenting cells (APC) also plays a role, skewing T cells via the IL-12 type mechanism (i.e. Th1 response) towards a good antiviral response, with a faster recovery of disease, or towards an IL-10 type of response (i.e. Th2 response) and recurrent wheezing. In this study, the group I zafirlukast pre-treated sensitized animals had a significantly lower BAL fluid IL-4 and IL-10 cytokine levels and lung tissue IL-4, IL-5 and IL-10 mRNA levels than the group II OA-treated BNR, suggesting an indirect mechanism of zafirlukast on Th2 response with a faster recovery of disease [22, 23].

IL-2, IFN- γ and TNF- α are Th1-related cytokines. Other cellular sources of IL-2 are cytotoxic T cells, and natural killer (NK) cells; IFN- γ are other NK cells, and TNF-TN are other somatic cells. The principal cellular sources of IL-6 are activated Th2 cells, APC and other somatic cells. IFN- γ strongly up-regulates monocyte and macrophage functions and, importantly, is also a potent eosinophil-activating cytokine [24, 25]. TNF- α is considered an important proinflammatory cytokine that may play a role in the development of late-phase asthmatic reaction. Gosset et al. showed that production of TNF- α by macrophages after an antigen challenge was increased only in patients who developed a late asthmatic response [26]. Like TNF- α , IL-6 is a proinflammatory cytokine. TNF- α and IL-6 facilitate the development of inflammation by several mechanisms, including up-regulation of intercellular adhesion molecule (ICAM-1) expression [27] and potentiation of cysteinyl LT release by eosinophils [28]. iNOS has immunomodulatory effects. In this study, we found that the group I zafirlukast-pretreated BNR had a significantly reduced IL-2 and IFN- γ cytokines level in BAL fluid, and reduced lung tissue mRNA levels than the group II OA-treated BNR, suggesting that zafirlukast also has an immunomodulation effect on Th1 response and other immune-related cells.

The results in our study indicate that zafirlukast inhibited the expression of a very wide range of cytokines. The number of macrophages, neutrophils, lymphocytes and eosinophils in BAL fluid was also reduced after zafirlukast pretreatment. Although the lung tissue mRNA levels decreased after zafirlukast pretreatment, the possible mechanisms of the effects of zafirlukast are very unlikely to be at the transcriptional level. The possible mechanisms are perhaps blockade of LT-induced chemotaxis and/or adhesion mechanisms (P-selectin or integrin expression), and/or reductions in vascular permeability. Increased apoptosis of neutrophils [29] and eosinophils [30] was also reported. All of these mechanisms can result in reduced recruitment and decrease the number of macrophages, eosinophils and T lymphocytes, and inhibition of the release of proinflammatory cytokines TNF- α , IL-6, IL-12 and IFN- γ , and inhibited the expression of both Th2 and Th1 related cytokines and inflammation. Calhoun et al. evaluated the effect of zafirlukast in 11 allergic asthmatic patients. They found that zafirlukast altered cellular infiltration and activation associated

with antigen challenge [28]. Other studies have also indicated that zileuton, a 5-lipoxygenase inhibitor [31], and cysteinyl leukotriene antagonist [32] reduced airway inflammation in a manner similar to our findings.

In conclusion, we have demonstrated a protective effect of zafirlukast against late-phase bronchoconstriction and inflammation induced by OA in BNR. Our study results support the theory that the drug exerts this effect at least in part by affecting proinflammatory cytokines, T cell-related cytokines and iNOS production.

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